FEASIBILITY OF BIOLOGICAL INDICATORS AS MEASURES OF COMMUNITY EXPOSURE TO ORGANIC AIR CONTAMINANTS

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l'exposition de la population aux contaminants atmosphériques organiques"

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ABSTRACT

The use of biological monitors (blood, urine, milk, adipose tissue, breath, saliva, nail clippings and hair) to follow the uptake by and incorporation in the body of organic pollutants as reported in the literature from 1960 to the present is reviewed.

Trace elements and organic residues detected, the concentration levels at which they have been detected, and the analytical techniques employed are given for each matrix material. In the authors' opinion, prospects for the use of biological monitors to assess community exposure to airborne contaminants are limited: by the virtual impossibility of distinguishing between absorption of the contaminant by inhalation, by ingestion, or through the skin; by the very short biological half-life of many contaminants, and by the difficulty of distinguishing the organic contaminant in the complex biochemical constitution of the matrices.

158 References.

ABBREVIATIONS USED IN THE REPORT

OCP's	organochlorine pesticides
PCB's	polychlorinated biphenyls
DDT	1,1,1-trichloro-2,2-bis(p-chlorophenyl)ethane
DDE	1,1-dichloro-2,2-bis(p-chlorophenyl)ethylene
BHC	benzenehexachloride (1,2,3,4,5,6-hexachlorocyclohexane)
HCB	hexachlorobenzene
PBB's	polybrominated biphenyls
DDD	1,1-dichloro-2,2-bis(p-chlorophenyl)ethane
VCM	vinyl chloride monomer
PCT's	polychlorinated terphenyls
HEAA	hydroxyethoxyacetic acid

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1. INTRODUCTION

Human and animal populations increasingly are being exposed to chemicals that are in the environment and are potentially harmful to their health. There are literally thousands of organic compounds with this potential which must be considered in environmental pollution studies: for example, polychlorinated and polybrominated biphenyls, halogenated hydrocarbons, and low molecular weight hydrocarbons. In many cases, the effects of these and other chemical contaminants are unknown; in other cases, certain compounds have been shown to be carcinogenic or to have harmful somatic effects. It is, therefore, of great importance to determine the precise amounts of these harmful and potentially harmful compounds taken up by humans and to establish areas, especially in occupational environments, where these compounds are expected to be present in dangerously high concentrations.

This report considers the use of biological monitoring to detect uptake dose and/or body burden of contaminants from the atmosphere. The literature from 1960 to the present has been searched. A survey of the pertinent references is given, together with suggestions for the directions that future work in this area might take.

2. BIOLOGICAL MONITORING

The use of blood, hair, urine, milk, and adipose and other tissues for monitoring has been widely employed to determine actual dose or body burden of several pollutants. While the technique has limitations, efforts are being made[1] to evaluate and confirm its usefulness in assessing environmental pollution by specific compounds.

Several problems and limitations are associated with the use of biological monitoring as established by Waritz[2] and Zielhuis[3] and these must be kept in mind when developing a biological monitoring program to be used to determine current exposure or body burden.

Biological monitoring which takes human beings as sampling units, creates certain degrees of inconvenience for the subjects. Loss of time and emotional unrest are two reasons people reject biological monitoring programs. Unions are beginning to object to biological monitoring in the workplace because of their concern about employees being used as "guinea pigs" and other management issues.

Routine biological monitoring of human subjects can only be carried out for a limited number of environmental pollutants. Contaminants which exert functional changes in organ functions, or which give rise to a subjective experience (smell, eye discomfort) do not provide a valid indication of exposure. Such compounds as SO_2, H_2SO_4, NO_x, and ozone are in the latter group of pollutants.

Many compounds (organochlorinated pesticides (OCP's), polychlorinated biphenyls (PCB's) or their metabolites) are persistent and can be stored in various human tissues and body fluids. Other compounds (organic phosphates, carbamate pesticides) are rapidly metabolized in the human body and eliminated. Still others (aliphatic hydrocarbons) are quickly eliminated without change.

Shafik[4] in studying human exposure to pesticides has obtained an index of biodegradability for some of these compounds. This index is based on the correlation between environmental stability and fat solubility. Halogenated compounds which are more fat soluble are more resistant to biodegradation, while methylated pesticides are more water soluble and, therefore, more biodegradable[4].

If external exposure is highly variable in time and if the biological half-life of the index chemical is relatively short, a biological monitoring program may fail in its objectives. Thus, various types of biological monitoring programs can be distinguished according to the duration of total exposure to be covered:

(i) Agents with a short biological half-life (< 10-15 hours) or which cause a short-term effect in the biological specimen examined. Here, biological monitoring indicates recent exposure (hours or days) to such components as chlorinated solvents or benzene.

(ii) Agents with a moderately long biological half-life in the biological specimen examined (up to 1-2 months). For example, biological monitoring of mercury and lead levels in blood and urine or hair will indicate exposure over the last several months.

(iii) Agents with a long biological half-life (months) in the assay material examined. In this case, biological monitoring of blood, adipose tissue and hair can indicate long-term, low-level human exposure to OCP's and other persistent pollutants.

(iv) Agents with a very long biological half-life or which cause persistent effects in the biological specimen examined. For instance, biological monitoring of lead in bones and teeth and of asbestos in lungs can indicate the, more or less, total lifetime exposure of the subject.

Thus, a knowledge of the biological half-life of the index chemical (metabolite or parent pollutant) in the assay material is esssential in establishing a biological monitoring program to assess human exposure to environmental pollutants. For example, it would be useless to monitor end-of-shift urine to estimate a worker's single day exposure to a pollutant if the index chemical does not appear in the urine sample until long after exposure.

The values of human urinary excretion half-life for several industrial pollutants as given by Waritz[2] are summarized in Table 1.

Table 1 - Human Urinary Excretion Half-life for Some Pollutants

Pollutant	Index Compound(s)	Excretion Half-life (hr)
Styrene	Phenylglyoxylic acid Mandelic acid	8.5 7.8
Toluene	Hippuric acid Hippuric acid	~ 4(α-phase)(a) ~ 12(β-phase)(b)
T_3CE	Total trichloro compounds	41
Phenol	Phenol	3.4
Xylene	Methylhippuric acid	3.8

(a) α-phase is the initial, rapid decay
(b) β-phase is the later, slower decay

In general, aliphatic and aromatic hydrocarbons are rapidly eliminated and the analysis of blood, urine and expired air is probably the most practical approach to evaluate current exposure. Organophosphorus and carbamate pesticides do not accumulate in the human body. Their ability to inhibit cholinesterases in blood and/or the concentration of their metabolites in urine are/is used to determine current exposure to these pollutants. Chlorinated solvents are quickly eliminated unchanged (in expired air) or as a metabolite (in urine). Their concentration in blood, expired air, and urine indicates the last few hours' exposure. Blood DDT levels indicate only very recent exposure to this pollutant. The presence of DDE in blood, however, reflects a chronic exposure to DDT[5]. A high increase in oral DDT intake does not affect the levels of DDE in blood.

Exposure to the volatile alcohols can be determined by analysis of expired air, blood or urine. These compounds are rapidly metabolized without accumulating in the organism. Therefore, an evaluation of a time-integrated cumulative exposure by tissue analysis is impossible. The exposure of humans to volatile ketones can be easily evaluated in expired air. No long-term accumulation of the most widely used ketones seems to occur in human tissues.

In general, there are three pathways by which contaminants can enter the body. These routes of entry are the mouth (oral), respiratory tract (inhalation), and the skin (absorption through the epidermis). Once the pollutant is incorporated into the bloodstream the route of entry cannot be identified. Therefore, biological monitoring will indicate only the total exposure to a chemical regardless of the pathway by which it was absorbed.

Although the determination of body burden of or total exposure to a chemical is usually desired, the use of biological monitoring to identify the source of pollution can be ambiguous since many contaminants can also be ingested in the diet. Thus, pesticide residues in fruit, PCB's and OCP's in cow milk, methylmercury in fish, and the residue of toxic metals in vegetables might eventually be incorporated into the human body. Therefore, the control group used to determine "normal levels" of contaminants in humans must be carefully chosen.

3. BIOLOGICAL MONITORING OF ORGANIC COMPOUNDS

Reviews of the use of biological monitoring to determine the actual dose or body burden for organic compounds have appeared[2,6,7]. Although biological monitoring does give an accurate value of the concentration levels of some contaminants in a specific tissue, several biochemical problems associated with this technique have been identified.

Among the general factors which must be considered in developing, applying, and interpreting a biological analysis should be[2]: metabolism, age and sex of the subject, normal levels of index compounds, and analytical methodology. Biological monitoring programs which resolve these problems while establishing standards for use by other researchers need to be organized. Based on the knowledge currently available, it is, however, feasible to conduct biological monitoring programs for some pollutants and human tissue[1].

The use of biological monitoring as a method of environmental control analysis should be supplementary to personal monitoring and to manual and automated area monitoring--the three most common techniques now used to monitor worker exposure to atmospheric contaminants.

Berlin et al.[1] have reviewed the biological specimens which may be useful for the assessment of human exposure to environmental pollutants.

The remainder of this section reviews some of the important books and articles on biological monitoring of organic compounds, excluding work on the monitoring of hair.

A. Blood Analysis

Chlorinated pesticides, polycyclic aromatics, phenol derivatives, alcohols, and other toxic substances are found in blood as a result of exposure to these chemicals. A blood analysis would then indicate the extent of such exposure. For example, a gas chromatographic technique has been used to determine PCB's taken up as a result of an occupational[8] and a non-occupational[9] exposure, and of ingestion of rice[10], and fish[11]. Residues of DDT and its metabolites have also been found in blood[12-19]. Significant levels of BHC and DDT were found in all milk and blood samples of nursing mothers[19]. Men occupationally exposed to HCB showed high levels of contamination[21].

High levels of the α, β and γ- BHC's in blood have also been found in people occupationally exposed to these contaminants[22]. Morgade et al.[23] have determined the level of polyhalogenated phenolic compounds in blood serum and adipose tissue resulting from ingestion of contaminated drinking water. Dogushi and Funkano[24-25] have estimated the residue levels of PCT's, PCB's and DDT in the blood of people <u>not</u> occupationally exposed to these compounds. Organic

mercury compounds[26-29], paraquat[30, 31], chloroform[32], and PBB's[33] also have been found in human blood. A relationship between total derived DDT in the blood and in the fat of residents of two locations in Ontario was obtained by Brown and Chow[20].

Besides the direct method of determining human exposure to pollutants by using pollutant concentration in blood, another, indirect, method applicable to organophosphorus and carbamate insecticides exists. This method measures the depression of blood cholinesterase activity due to the presence of these anticholinesterase pesticides. Several studies[34-38] have been carried out applying this technique.

Some of the problems related to the use of human blood in biological monitoring to assess the extent of exposure to or the body burden of a specific contaminant are pointed out by Baselt[6]:

(i) Objections by the subject to frequent samplings;

(ii) low concentration of contaminant in blood with concomitant difficulty in analysis;

(iii) transient concentration fluctuations depending on the history of exposure;

(iv) possible interference in the analysis when anticoagulant is needed;

(v) selection of blood containers and collection devices so as to avoid contamination.

Some of the analytical procedures for blood analysis published by Baselt[6] are summarized in Table 2.

B. Urine Analysis

A large fraction of the chemicals encountered in the environment and taken up by the human body is eventually excreted in the urine as metabolites. Therefore, by monitoring levels of urinary metabolites, the index of exposure to these contaminants can be established.

Berlin et al.[1] indicate that correlations between the body burden or personal exposure and level of concentrations in urine of environmental pollutants such as phenoxy herbicides, pentachlorophenol, chlorinated solvents, highly volatile non-substituted aliphatic and aromatic hydrocarbons, alcohols and organophosphorus esters have been established. Further research is required to confirm the utility of biological monitoring to obtain such correlations for DDT, polycyclic hydrocarbons, glycols, ethers, ketones, aldehydes, and phenol derivatives.

A few basic methods permit the monitoring of a large number of metabolites in urine[45-46] which may be classified into one of several broad categories: phenols, anilines, triazines, etc. Erickson et al.[47] have developed an analytical methodology using gas chromatography suitable for monitoring residues of the s-triazine herbicides and their metabolites in human urine. Pentachlorophenol[48], chlorinated phenol metabolites of HCB[49], and chlorobenzilate[50] residues

Table 2 - Analytical Procedures for Organic Compounds in Blood

Compound	Method	Sensitivity	Comments Interferences	Reference
OCP'S: aldrin, BHC, DDT, DDE, dieldrin	OCP's metabolites are extracted from plasma with hexane, analyzed by GC-ECD with double aluminum column: 1-10% DEGS on chromosorb G, 2-5% DOW 200 on chromosorb G	0.1 µg/L	Double column increases specificity, no interference reported	40
Chlordecone (Kepone)	Benzene extracts chlordecone from blood. Solution is analyzed by GC-ECD with glass column: 10% DC-200 on Gas Chrom Q support	10.0 µg/L	Since extraction procedure is relatively specific for chlordecone, no interference found	41
Hexachlorophene	Hexachlorophene is extracted from blood with ether/ethanol and derivatized with acetic anhydride. It is then analyzed by GC-ECD with glass column: 3% OV-225 on 80/100 Supelcoport support	10 µg/L	No interference reported	42
Carbaryl	Blood is incubated with acetylcholine solution, hydroxylamine is added and the absorbance is read at 540 nm	0.2 µ mol	Erythrocyte cholinesterase activity is less variable than plasma esterase activity	43
PBB's	Hexabromobiphenyls are extracted from serum with hexane/ether and analyzed by GC-ECD with glass column: 1% OV-101 on Gas Chrom Q	>100 µg/L	No interference reported	44
PCB's	PCB's are extracted from plasma with hexane. Extract is purified and then analyzed by GC-ECD with glass column: 2% OV-1 on Gas Chrom Q	>100 µg/L	Several OCP's have similar retention times	25

in urine also have been detected using a gas chromatography technique. Gas chromatographic analysis of urinary alkyl phosphate metabolites or organophosphorus pesticides has been reported by several authors[34,51-54].

Some of the problems which arise when urine is used as a biological monitoring medium are:

(i) The index chemical can arise from diet or other non-environmental sources;

(ii) the index chemical may be a metabolite of several compounds leading to erroneous conclusions;

(iii) the natural levels of the index chemical may be so high and so variable that they invalidate the technique for the exposure levels of interest;

(iv) urine analysis over 24-hour periods, though desirable, is impractical;

(v) the sample, if not analyzed immediately, should be refrigerated or frozen until time for the analysis;

(vi) standardization of the analytical method and report forms is needed to avoid inconsistencies.

In addition to the information on analytical procedures given above, further methods of analysis for organic compounds in urine are summarized in Table 3.

C. Human Milk Analysis

Mother's milk is also a body fluid where pollutant residues have been found. This fluid, which carries contaminants as an excretory pathway of the human body, is thus a source of contamination for breast-fed neonatals.

The presence of PCB's, DDT and other OCP's in the human milk of the North American population has been reported[60-66]. OCP residues in human milk have also been found in Portugal[67], Guatemala and El Salvador[68], Japan[69], Norway[70] and in several other countries[64].

Polishuk et al.[64] have reviewed the determination of organochlorine compounds in human blood and milk. The concentration of DDT, BHC, Dieldrin, heptachlor expoxide, HCB and PCB's in the human milk of the population of several countries is also reported in this article.

Three general steps comprise the procedure for analysis of human milk to determine pesticide residues: lipids extraction from milk; pesticide extraction from lipids; analysis of extracted pesticides.

Table 3 - Analytical Procedures for Various Organic Compounds in Urine

Compound	Method	Sensitivity	Comments	Reference
Benzidine	Benzidine is extracted from urine with ether. A back-extraction is made into dilute acid. The solution is then analyzed by LC-ECD. Column SS: Micro bondapak C_{18}	<1 µg/L	Detector operated at 450 mv against Ag/Ag Cl reference electrode. No interference known.	55
Diazinon	Organic phosphate metabolites are extracted from urine with ether. Reagent is added and absorbance is measured at 320 nm	0.2 µg/L	Several pesticides are metabolized to these phosphate metabolites. Use of a control urine as a blank for the reference cell is suggested.	56
Dioxane (as HEAA)	Methyl derivative of β-hydroxyethoxyacetic acid is formed by heating in the presence of HCl and CH_3OH. After completion of reaction the solution is analyzed by GC-FID with glass column: 3% OV-17 on Supelcoport	50 µg/L	Broad peaks after some time. Change ends of column packing occasionally.	57
Paraquat	Paraquat is separated from urine in a cation exchange column, and eluted from the column with NH_4Cl, after adding reagent absorbance is measured at 390, 394, and 398 nm	10 µg/L	No interference is known	58
Parathion	p-Nitrophenol is liberated from urine. The released compound is extracted with benzene/ether. Analysis by GC-ECD with glass column: 5% DC-200 on Gas Chrom Q	50 µg/L	Method is relatively specific for p-Nitrophenol. p-Nitrophenol is also a metabolite of methylparathion.	59

Brevik[71] and Strassman et al.[66] have used a gas chromatograph equipped with tritium electron capture detector to determine the level of organochlorine compound residues in human milk. The lower limits of detectability for some compounds as given by these authors[66,71] are shown in Table 4.

Table 4 - Limits of Detectability for Organochlorine Compounds in Human Milk

Compound	Limit of Detectability (ppb)
HCB	0.1(a)
	10
DDE	2(a)
	10
DDD	2(a)
DDT	2(a)
	20
BHC (lindane)	10
Endrin	20
Aldrin	10
Dieldrin	10
PCB's	1000

(a) from Brevik[71], the rest from Strassman et al.[66].

Berlin et al.[1] have indicated that, based on the experimental results so far obtained, human milk is a suitable biological monitor for the determination of the body burden in DDT, PCB's and OCP's.

A variety of about 20 other trace elements has been measured in human milk by atomic absorption and neutron activation analyses.

Some of the problems associated with the use of human milk as a biological monitor to determine exposure or body burden are:

(i) Limited occurrence (the method is not universally applicable as are the blood and urine methods--lactating women are a very small subgroup with a narrow range of ages and may not be representative of exposure among the population at large);

(ii) low concentration of contaminant in the fluid;

(iii) contamination occurs very easily during sample collection;

(iv) the sample must be kept frozen until the analysis is performed.

D. Adipose Tissue Analysis

Several persistent pollutants have been found in human adipose tissue. In fact, adipose tissue is estimated to be the most suitable indicator specimen for monitoring the levels of OCP's[1]. Data are available showing a very uniform distribution of these compounds in the fat from different sites in the body.

The presence of PCB and OCP residues in adipose tissue of Canadians has been studied by Mes et al.[72]. In this study (1972) a lower DDT level compared to that found previously (1969) was reported. This phenomenon could be attributed to the restricted use of this pesticide in Canada since 1969.

Electron-capture gas chromatography has been used to determine PCB[73], OCP[74,75] and OCP and PCB[76] residues in human adipose tissue.

Neutron activation analysis and electron-capture gas chromatography were used to determine HCB, PCB and DDE in liver and adipose tissue of Norwegians[77].

Polycyclic aromatic hydrocarbon residues were determined in human fat and liver[78]. A high pressure liquid chromatograph equipped with a reverse-phase column and a fluorescence detector has been used to measure down to ppt levels. The detection limit to benzo(a)pyrene is 10 pg.

One of the most serious limitations to using adipose tissue for biological monitoring is the need to perform a surgical operation on the subjects sampled.

As with almost all biological materials, adipose tissues must be kept frozen until the analysis is performed.

E. Miscellaneous

1. Breath

The use of breath analysis to detect toxic gases and volatile organic compounds is widely accepted. This method possesses the precision for establishing an unequivocal diagnosis of exposure or body burden for pollutants such as chlorinated solvents, highly volatile non-substituted aliphatic and aromatic hydrocarbons, alcohols, ethers, ketones and carbon monoxide[1,2,79].

Different analytical techniques which are used in breath analysis are described by Linch[7]. The sensitivity of gas chromatography and/or infrared spectroscopy analyses of some environmental pollutants is given in Table 5.

Table 5 - Analysis of Gases and Vapors in Expired Air

Compound	GC Sensitivity (ppm)[a]	IR Sensitivity (ppm)[a]	IR Sensitivity (ppm)[b]
Acetone	0.1	5	5
Benzene	0.1	20	20
Carbon Monoxide	0.01	5	20
Chloroform	0.01	1	1
Diethyl Ether	0.1	5	5
Methanol	0.2	5	5
Turpentine	0.4	10	10

(a) from Stewart (79)
(b) from Linch (7)

As has all biological monitoring, breath analysis has its own limitations and problems which must be kept in mind when using it to determine exposure to environmental pollutants:

(i) The method is limited to highly volatile compounds:

(ii) the rapid excretion of most volatile substances limits breath sampling to a relatively short time after exposure;

(iii) a standard collection device must be used and care must be taken to avoid losses by permeation or adsorption;

(iv) the samples should be stored at the constant temperature of the exhaled breath (37°C) to avoid condensation of water vapour and to eliminate physical or chemical alteration of the initial constituents of the sample.

2. Saliva

Although very little work has been done on the use of saliva for biological monitoring, saliva analysis has demonstrated some utility for following the blood levels of drugs(2).

Olmez et al.(82) have confirmed previous observations relating taste sensory disorder to low concentrations of zinc in saliva. They have used instrumental neutron activation analysis, INAA, and instrumental photon activation analysis, IPAA, to measure concentrations of Na, P, K, Cl, Cr, Fe, Co, Zn, Se, Br, Rb, Sb, Cs, Ca, and I.

Quantitative evaluation of mercury levels in human saliva of unexposed and exposed populations has been reported by Joselow et al.(83). Mercury was not found in the control group (unexposed) but appeared at levels ranging from 2 to 12 µg/100 mL of saliva in the exposed group.

Berlin et al.(2) have summarized the usefulness of more than 35 tissues and body fluids which may be used in biological monitoring for trace elements or organic pollutant compounds. In some cases there is enough evidence of the suitability of the tissue to indicate the body burden for some contaminants, while in other cases more experimental work is necessary to arrive at definite conclusions.

3. Nail Clippings

Since their composition is similar to that of hair, nail clippings can be used to determine trace element levels in the human body. Jervis et al.(80) using a neutron activation technique determined the concentration of 12 elements. Eighteen elements have been analyzed in nail clippings by Cooper and Langford(81) using an X-ray fluorescence technique.

4. HUMAN HAIR IN BIOLOGICAL MONITORING

Human hair has been and continues to be the subject of analysis of several research groups that are involved in determining the level of exposure of humans to environmental pollutants.

Scalp hair, because of its continual growth, offers the researcher the unique possibility of determining both the actual body burden and the history of uptake of contaminants.

Structure, growth and trace element sources characteristic of hair have been covered by different authors[84-86]. In this report, only a general review is given.

Hairs are threadlike epithelial fibers, mainly, 85 to 93%, composed of keratin, a polymerized protein. Water makes up 4 to 13%, lipids 2.3% and the ash residue constitutes 0.23 to 0.8% of the mass[87]. The hair shaft is divided into three major regions, a strong exterior layer (the cuticle), a thick wall (the cortex) and a central canal (the medulla).

The growth of an individual hair is cyclic rather than continuous, periods of growth alternating with periods of rest. The length of the cycle and the rest/growth ratio vary considerably depending on the species and on the region of the body[85]. A typical rate of growth for human head hair is about 0.35 mm per day.

The hair shaft is exposed to several potential sources of trace elements besides the blood stream which discharges these substances into the growing hair. The sebaceous gland is a source of trace elements derived from body tissues. Eccrine sweat glands secrete a predominantly aqueous solution and they are also a continuing source of trace elements derived from body tissues. Apocrine sweat glands which are confined to axillary, pubic, and perianal areas secrete a milky fluid, which is rich in lipids.

All of these trace element sources represent direct pathways from the diet to the hair structure, in distinction to exogeneous materials containing trace elements to which the hair may be exposed, such as water, soaps, shampoos, oils, lacquers, dyes, air, etc.

A. Sampling of Hair

The variety of sources of trace elements in hair makes a universally applicable definition inadequate for the purpose of establishing "normal" population hair contents of trace elements. The International Atomic Energy Agency has recommended that hair samples should represent a statistically random sampling of 1000 or more individuals from the subject population and should consist of 100 strands per person taken from 10 to 20 different places on the head. The normal values of trace elements in hair for each country should be taken by sampling people from over the entire country.

Valkovic[86] has reviewed the study of trace elements in hair as related to hair colour, sex and age indicating the normal levels of some trace elements. Chuang and Emery[88] have also reported normal levels of trace elements in human head hair. Pankhurst and Pate[84] give the most complete summary of normal concentrations of trace elements in human hair of people living in different countries and around the world. (Actually, few really significant variations have been noted between races and countries, taking environmental and occupational perturbations into account.)

B. Washing of Hair Samples

From the analytical point of view, the composition of hair can be affected to some extent by its treatment before the analysis. Since hair may be externally contaminated by the application of shampoos, cosmetic preparation, atmospheric fallout, and other causes, many investigators suggest that hair samples should be washed prior to analysis.

The various washing techniques that have been reported range from the use of pure distilled water[89] to that of ethyl-enediaminetetraacetic acid[90,91]. Most researchers use mild washing techniques which do not substantially affect the composition of the elements chemically bound to or incorporated in the hair but which remove fat and dirt. Thus Jervis et al.[92-95] have washed their hair samples with doubly distilled water, absolute ethanol, and diethyl ether thereby removing oils, lacquers and externally attached dust particles. No appreciable differences between washed and unwashed samples in the elemental content of their hair was obtained. Barlow and Kapel[96] used a detergent-acetone washing technique which altered considerably the hair compositions. Chuang and Emery[88] in their study used water-acetone to wash the hair samples.

Phenomena which alter the true concentration of trace elements in hair due to body secretions, removal from hair in the washing process and the absorption of elements from exogenous sources, have given rise to a certain amount of discussion among researchers. Experiments on the absorption of elements by hair have led to different views. Thus Yoon[97] using a radiotracer technique has concluded that As, Pb and Hg are not readily incorporated into the hair structure. However, the data of Bate[98] show a different pattern indicating that Fe, Co, Cu, Zn, Ce, Br, Se and I can be absorbed by hair in different amounts depending on solution pH. The latter results have also been confirmed by other researchers[99-102]. Although Na and Mn have been found to penetrate easily into hair, Van den Berg et al.[103] have found that Cu, Zn and Cr ions reacted more slowly. They also noted that a part of the hair (medulla) is more difficult for ions to penetrate. Thus the trace element composition of this part might be an indication of the body burden at the hair growth time.

C. Digestion of the Hair Samples

Depending on the analytical technique to be applied, the hair sample may or may not need to be digested. Thus Barlow and Kapel[96] using atomic absorption spectroscopy for the determination of Cu, Ca, Fe, Mg and Zn have digested the hair sample with concentrated sulphuric acid and hydrogen peroxide. The same authors[96] have prepared a base-digested sample of hair using soluene-350, a quaternary ammonium hydroxide, to determine Pb, Mn, and Cd concentrations, also by atomic absorption spectroscopy.

Chattopadhyay et al.[104] have digested hair samples using fuming nitric acid with the addition of an As, Zn, Cd and Hg carrier solution. This solution was left for about 16 hours in an ice bath to allow cold digestion. Subsequently hair samples were completely dissolved by the dropwise addition of concentrated hydrochloric acid to a heated mixture. After the hair strands were completely dissolved, an ion exchange column was used to separate the elements whose concentration was then determined by neutron activation analysis.

Abdel Aal et al.[105] have used a mixture of concentrated sulphuric acid and hydrogen peroxide to digest hair samples. Flame photometry was used to determine Na and K, and a colourimetric procedure was used to estimate Cu, Fe, P and Ca concentrations.

D. Analytical Methods

Several analytical techniques have been applied to determine trace elements in hair. Valkovic[86] has described the atomic absorption, neutron activation, X-ray emission and mass spectroscopy techniques which have been used extensively.

The concentration of mercury and other elements in human hair has been determined by several authors[27, 106-110] using atomic absorption spectrophotometry and instrumental neutron activation analysis.

Neutron activation analysis techniques have been applied[92-95, 104, 111-114] to determine several trace elements in hair. Chattopadhyay et al.[94] investigated several nuclear methods applicable to hair analysis: Instrumental Neutron Activation Analysis (INAA), Radiochemical Neutron Activation Analysis (RNAA), Intrumental Photon Activation Analysis (IPAA), and Radiochemical Photon Activation Analysis (RPAA). Fourteen elements included in standard reference materials supplied by the U.S. National Bureau of Standards, were analyzed using these techniques. The agreement between the reference values and those obtained by these experiments was excellent.

Although with less frequency than the previous methods, emission spectroscopy[115-117] and mass spectroscopy[118] have been used.

Other analytical techniques have also been used to determine trace element levels in human hair. Thus Barlow and Kapel[96] have determined S concentration as sulphate by titrating the solution with barium perchlorate. Abdel Aal et al.[105] have employed flame photometry to determine Na and K, and a colourimetric technique to estimate Cu, P, Ca, and Fe concentrations. Gas chromatography has been used by Matthews et al.[119] to determine the concentration of chlorinated hydrocarbons in hair.

E. Elements Detected in Human Hair

The 60 some elements found in human hair so far[84] are shown in Table 6.

Table 6 - Elements Dectected in Human Hair

H	Na	K	Fe	As	Pd	Ba	Tl	Th
Li	Mg	Ca	Co	Se	Ag	La	Pb	U
B	Al	Sc	Ni	Br	Cd	W	Bi	
C	Si	Ti	Cu	Rb	Sn	Sr	Po	
N	P	V	Zn	Sr	Sb	Pt	Ce	
O	S	Cr	Ga	Zr	I	Au	Sm	
F	Cl	Mn	Ge	Mo	Cs	Hg	Eu	

There is no definitive answer to the question of precisely which elements can be found in hair or in what form these elements are attached to or incorporated in the hair structure. The high content of heavy metals in hair compared to those levels found in blood

(Hg hair/Hg blood ≈ 300 (121-123),

and

Pb hair/Pb blood ≈ 100 (95))

is probably due to a very stable S-metal bond occurring during hair formation. However, such high ratios indicate that a less sensitive analytical method can be used for hair compared to blood. As well, sampling is less intrusive to the human subjects being monitored.

F. Dectection of Organics in Hair

Matthews et al.[119] have shown that lipids in the hair structure form an excretory pathway for chlorinated hydrocarbons (DDT, PCB's, Dieldrin). Chlorinated hydrocarbons were extracted from hair with petroleum ether. After cleaning the samples, DDT and its derivatives were oxidized to dichlorobenzophenone using chromic acid as described by Miles[120]. Dichlorobenzophenone was separated from PCB compounds and both fractions were analyzed by gas chromatography. Chlorinated hydrocarbon concentrations found in human hair in ppm were: DDT from 0.01 to 0.10; Dieldrin, 0.01 and lower; and PCB's from 0.34 to 0.76. Halides concentrations can also be measured to ppm levels and below by neutron activation analysis.

Asada et al.[124] have found that PCB concentrations in hair were higher than those in blood and have noted a possible correlation between the PCB concentrations in these two specimens. They have also found that the hair PCB concentrations in normal and PCB poisoned subjects was very close. The middle-aged group showed a higher content of PCB's in their hair than did youths. However, no sex difference was noted.

Takahashi et al.[125] have also detected organochlorine compounds in human hair and milk. Using gas chromatography in their analysis they have reported that PCB's were detected in 77% of the milk samples and in all hair samples from the residents of Sendai, Japan in 1975-1976. The average total PCB's level was 0.008 ppm for milk samples and 0.31 ppm for hair samples. They noted that females in their population sample showed a lower content of PCB's in hair, but a higher content of total DDE. The average total DDE concentration found was 0.039 and 0.31 ppm in milk and hair respectively.

The high value of the ratio PCB's hair/PCB's milk (≈40) found by Takahashi et al.[125] cannot be fully explained until more is known about the analytical procedures followed by these authors. For example, the result for hair may be based either on whole hair or on the lipids extracted from the hair, while the result for milk may, similarly, be based either on whole milk or on the lipids extracted from the milk. If the PCB contents were determined on the same basis, the high value of the ratio can be explained as the result of exogenous sources of contamination which increase the PCB residues in hair. However, if the results were determined on different bases, the high value of the ratio may be the result of this incongruency.

Although no evidence has been reported, we suspect that, since PCB's, DDT, DDE and Dieldrin have been detected in hair, other lipophilic, persistent organic compounds, such as PBB's, HCB, HBB, BHC and organochlorine pesticides in general might be excreted in the lipid content of the hair. Furthermore, the level of these pollutants in the hair should be correlatable to the body burden of these contaminants.

Since compounds like glycols, amino and nitro derivatives, alcohols, ethers, ketones, aldehydes, amides and volatile non-substituted aliphatic and aromatic hydrocarbons are rapidly metabolized and/or eliminated they do not accumulate in the lipid content of hair[1]. Thus it is not likely that hair can be used for biological monitoring of these pollutants.

Although the number of organometallic compounds which are known to be environmental contaminants is small, these compounds may create important toxicological problems. Since cysteine residues in the hair structure can stabilize metal alkyls by forming S-metal bonds, hair analysis may be useful in determining the extent of contamination by compounds such as Pb, Hg, As and Sn alkyls. Several workers[106, 107, 121, 122] have reported methyl Hg levels and total Hg in human hair using neutron activation analysis.

5. NON-BIOLOGICAL AIR MONITORING

Personal monitoring and stationary area monitoring are two non-biological monitoring methods widely used in envirpnmental control programs.

Although stationary area monitoring provides an accurate measurement of the contaminant concentration in a particular spot, it does not provide a good measurement of human exposure to airborne pollutants. This is mainly due to population mobility and activity patterns. People move among areas with different levels of contamination and inhale more or less air according to their activity. Thus personal monitors have advantages over the stationary area monitor, since they follow these activity patterns and local distribution of airbone contaminants.

Lee and Mage[126] have reviewed the use of personal exposure monitors as an environmental control technique which has become widely applied in industrial hygiene programs. The reduced size, portability, low cost and easy operation of these sampling devices are noted by these authors who also indicate the main limitations of these samplers, that is, their low specificity and sensitivity.

Several personal monitoring devices have been developed in the last few years. These are listed in Table 7, including some samplers recently reported by Lee and Mage[126].

Table 7 - Recent Advances in Personal Environmental Monitoring

Pollutant	Monitor Feature	Active/Passive	Reference
Acrolein	Diffusion	P	(127)
Ammonia	Diffusion	P	(128)
Benzene	Diffusion	P	(127)
Arsenic	Tape	P	(128)
Carbon monoxide	Diffusion	P	(149)
Hydrogen chloride	Ion Electrode	P	(127)
Lead	Tape	P	(128)
Phosgene	Tape Sampler	A	(128)
Sulfur Dioxide	Ion Electrode Tape Sampler	P A	(128)
VCM	Diffusion	P	(127,128)

The presence of pesticides, PCB's and other organic residues in air is a matter of major concern among researchers working in environmental programs.

Davis[129] has designed and evaluated a procedure for personal monitoring related to the use of pesticides. Different methods for the assessment of dermal, hand and respiratory exposure to these compounds have been presented by this investigator.

Lewis[130] has described several sampling techniques used to determine pesticide concentrations in air. Similar information is given by Van Dyk and Visweswariah[131]. Four types of samplers have been described:

accumulative samplers,
reactive samplers,
continuous and sequential samplers, and
grab samplers.

There are several types of accumulative sampling devices commonly used to collect air samples. Solid sorbents, due to their capacity for adsorption and small size, are the active component in some of the most popular accumulative sampling devices.

Polyurethane foam has been used by several authors[132-136] as a collector medium for chlorinated biphenyls, polychlorinated naphthlenes, OCP's and organophosphorus pesticides and other herbicides. Porous polymer adsorbents have also been used to collect organic air contaminants[137-139].

Techniques using wet impingers for sampling air contaminated with pesticides have been developed by Miles et al.[140]. Pesticide dusts and vapours are trapped more efficiently from air by Greenbury-Smith type impingers and by Anderson and Llugren cascade impactors than by filters.

Several personal monitors for organic and inorganic pollutants in the workplace have been developed in recent years[130]. Active samplers use a pump to draw the contaminated air through the adsorbent, while passive dosimeters are based solely on the diffusion of the pollutant from the air to the surface of the sorbent.

Melcher et al.[141] have developed an active sampler for Chlorpyrifos and other pesticides which uses a chemically bonded sorbent. Hill and Arnold[142] have designed a personal monitor for pesticides using a cellulose ester filter and Chromosorb 102 as collector medium. Their device uses a portable pump to sample the air.

Passive dosimeters for monitoring organic vapour concentrations in workplace air have been developed recently by the 3M[143] and MSA[144] companies.

The analysis of the collected samples is usually performed using gas chromatography techniques. Thus, the organic vapours trapped on activated charcoal are desorbed (thermally or by CS_2) and analyzed in a gas chromatograph with a flame ionization detector[143, 144].

Benzo(a)pyrene and other polynuclear arenes are usually detected in trace amounts by liquid chromatography, HPLC and reversed phase liquid chromatography with fluorescence detection[145]. Green et al.[146] have developed a gas phase spectrophotofluorometric technique to analyse polynuclear aromatic hydrocarbons. The minimum detectable amounts in a 3 mL injected solution for benzo(a)pyrene, benzo(e)pyrene, benzo (k) fluoranthene and perylene are 1.6×10^{-8} g, 9.9×10^{-8} g, 1.8×10^{-9} g and 6.0×10^{-8} g respectively.

The analysis of PCB's and the analysis of pesticide residues in the presence of PCB's are extensively reviewed by Krull[147] and Reynolds[148].

Human subjects may be exposed to a single contaminant through several sources such as air, food, water or drugs, and by various pathways (respiratory tract, skin, intestinal tract) making it impossible to measure the total exposure from external sources in a direct way. Therefore, environmental monitoring using area or personal monitors should be supplementary to biological monitoring in order to obtain an accurate measurement of the dose or body burden. Conversely, biological monitoring may be inadequate if a particular source of the contaminant (e.g., ambient air) is to be identified. The need must be stressed for controls from the general population (selected rural groups, far-north populations, etc.,) to establish baselines by which to evaluate contaminant levels determined.

6. CONCLUSIONS

The following conclusions can be drawn with regard to the use of biological monitoring for the assessment of human exposure to environmental contaminants.

In spite of its limitations, the analysis of biological specimens has proved to be a useful tool for the determination of personal exposure or body burden for some specific pollutants. The following biological specimens can indicate:

Blood - chlorinated pesticides, polycyclic aromatics, phenol derivatives, alcohols, PCB's, PBB's, carbon monoxide, organophosphorus and carbamate pesticides, heavy metals (e.g., Pb, Hg).

Urine - pentachlorophenol, chlorinated solvents, non-substituted aliphatic and aromatic volatile hydrocarbons, alcohols, organophosphorus pesticides.

Milk - organochlorine pesticides, PCB's.

Adipose tissue - organochlorine pesticides, PCB's polycyclic aromatic hydrocarbons.

Breath - chlorinated solvents, non-substituted aliphatic and aromatic volatile hydrocarbons, alcohols, ethers, ketones, carbon monoxide.

Although reports of the use of hair analysis to determine the body burden or exposure for organic compounds are few in number, these reports confirm that the lipid content of human hair is an excretory pathway for persistent organochlorine compounds.

Since human hair may be contaminated by exogenous materials, it is very important to evaluate the significance of these other sources which can otherwise lead to erroneous conclusions.

Human hair has the property of separating and concentrating trace elements from the blood stream and of averaging over longer periods than blood for chronic exposure and longer biological lifetime components. Therefore, organometallic compounds such as Pb and Hg alkyls can be retained in the hair structure allowing subsequent detection. Several researchers have determined methyl Hg concentrations in human hair which are inherently much higher than those found in blood.

Since compounds such as alcohols, ethers, ketones, aldehydes, glycols, non-substituted aliphatic and aromatic volatile hydrocarbons are rapidly metabolized and/or eliminated, they do not accumulate in human tissues. Therefore, the use of hair analysis to monitor these compounds does not appear possible.

Since the pollutants taken up by the body from several sources and through several pathways are absorbed into the blood stream and distributed throughout the body, biological monitoring cannot indicate the source of contamination unless other non-biological monitoring programs are also in place.

7. FUTURE PROSPECTS

Based on this examination of the literature it is our opinion that future prospects for the use of biological monitors (and particularly hair analysis) for the assessment of community exposure to organic air contaminants are limited. Two fundamental limitations exist: inability to distinguish between air and other sources of the contaminant and the analytical difficulty of distinguishing between the compound of interest and the many others in complex biological matrices such as hair. Other problems arise because of the limited persistence in biological tissues of many low molecular weight, volatile organic species of interest and the unfavourable partitioning of water-insoluble organic compounds in readily accessible tissues (blood, urine, hair). While these latter problems can be overcome by appropriate design of the sampling protocol to match the contaminants of particular interest and by using sophisticated analytical procedures, the former limitations are more difficult to surmount.

If the purpose of the monitoring program is to assess the exposure of individuals in the general public to air contaminants, then personal monitoring using non-biological methods (e.g., passive dosimeters) is a superior method (see Section V). There is no confusion as to the source of the pollution in this case and the proper conclusion can be reached regarding the validity of alternative sampling methods (e.g., area samplers) or the appropriate control method. However, biological monitors determine body burden, or the accumulated amount of a contaminant, regardless of its source. Biological monitoring can not in general distinguish between absorption by inhalation and absorption by ingestion. For many pesticides, for example, the latter route of absorption may be more significant. Measurement of blood or other tissue levels would then be a measure of dietary intake rather than of the time-weighted average concentration in air. Only by proper choice of a control group with the same diet but negligible ambient exposure history, at least to the contaminants of interest, can useful information be obtained. Unlike the use of biological monitors for workplace exposure, where the worker can be used as his own control, the natural differences between the control and test group may easily outweigh the expected differences in contaminant body burden. Obviously, the sample population size becomes more critical in the community exposure program.

Although hair analysis is well developed for determining community exposure to toxic metals, there were only three reports of the detection of organic compounds in hair. Unfortunately, hair, like other biological media, is an extremely complex sampling matrix which effectively masks the presence of low levels of specific organic compounds. Sophisticated separation and detection methods are necessary if the concentrations of organic compounds are to be determined in biological media. Some such methods are available for blood and urine analyses but

it is unclear that the sensitivity of these assays is sufficient for determining the typically much lower levels of less persistent compounds associated with community exposures (i.e., of contaminants other than pesticides).

It is conceivable that analytical procedures could be devised to detect various organic compounds in hair. Non-destructive INAA can determine total chloride which might be a useful (quick and simple) screening test. As another example, thermal desorption techniques applied to gently washed hair may be a means of recovering a sample of organic compound without having to digest the hair in acid or to subject the hair to extensive washing. Washing to remove external contaminants may also extract the lipid content of hair leaving nothing to be analysed. Both digestion and washing steps can result in significant losses of organic material or lead to significant changes in organic composition that would thoroughly obscure the intended analysis. Of course, the thermal desorption program that would be appropriate for activated charcoal passive dosimeters or other samplers would have to be modified to be appropriate for hair analysis. Extensive controls would be necessary to compensate for background effects. The resulting desorbed vapour could then be separated by capillary column gas chromatography and detected with the appropriate detector (electron capture, photoionization, etc.). It would probably be desirable to use GC/MS and/or GC/Ir techniques to identify the effluent from the GC to facilitate the qualitative analysis. Such techniques could be developed if it were felt that biological monitoring of organics using hair would be a useful adjunct to nonbiological personal monitoring programs.

In summary, it appears, at this point in time, that the likelihood of developing biological monitoring programs to determine and follow the up-take and incorporation in the body of airborne organic pollutants by the population at large is very small.

8. MONITORING OF NON-HUMAN BIOLOGICAL INDICATORS

Lichens, bird feathers and animal hair are among the non-human biological specimens which have been monitoried to assess the extent of environmental contamination.

1. Lichens

The presence or absence of certain common species of lichens which are particularly sensitive to environmental contamination and the lichen distribution pattern in a given area have been recognized for some length of time as related to degree and extent of pollution. The effects on lichens of pollutants such as sulphur dioxide, hydrogen fluoride and other air contaminants have been reviewed by Le Blanc and Rao[150], Gilbert[151] and Henderson[152]. James[153] has reviewed the effects of: (i) heavy metal fallout, especially Cu, Cd, Fe, Pb, Mn, Ni and Sn fallout; (ii) fallout of long-lived radionuclides; (iii) ionizing radiation; (iv) deposition of particulates from smoke and (v) fertilizers, fungicidal sprays and selective weedkillers.

Addison and Puckett[154] have related the accumulation of Al, K, S, Ti and V by certain lichens to both gaseous and particulate industrial emissions and to localized wind-blown dust.

2. Bird Feathers and Animal Hair

Bird feathers and animal hair have also been studied as biological indicators of environmental pollution. Johnels[155] has determined mercury levels in bird feathers and has related dramatic increases in these levels to the use of alkylmercury-containing pesticides. (Alkylmercury pesticide use commenced in 1940 in Sweden).

Benvenuti and Cerquiglini[156] have also been able to relate the degree of environmental contamination to lead levels in species living in a contaminated area. Thus, lead concentrations in the range 12-78 ppm were determined in chicken feathers from birds collected within 1400 m from a ceramic pigment factory, the suspected source of the pollution.

Ward and Brooks[157] have found a good correlation between lead levels in the hair and in the blood of sheep exposed to automotive emissions. They have also found that the washing treatment used in sample preparation is a critical step in the analysis of sheep wool.

Negishi[158] has determined residual levels of BHC, heptachlor, aldrin, dieldrin and total DDT in bovine feed, adipose tissue and hair by a GC-MS technique. The organic chlorine pesticide levels in the feed were very low - in the 2-42 ppb range. Low concentrations of BHC, heptachlor and aldrin and only trace amounts of dieldrin and total DDT were found in the adipose tissue samples. However, head hair samples showed very high levels of BHC (338-609 ppb) and heptachlor (119-293 ppb) but only trace amounts of the other pesticides.

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